EXHIBIT D

Induction of Tumors in Mice by Genomic Hypomethylation

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Genome-wide DNA hypomethylation occurs in many human cancers, but whether this epigenetic change is a cause or consequence of tumorigenesis has been unclear. To explore this phenomenon, we generated mice carrying a hypomorphic DNA methyltransferase 1 (*Dnmt1*) allele, which reduces Dnmt1 expression to 10% of wild-type levels and results in substantial genome-wide hypomethylation in all tissues. The mutant mice were runted at birth, and at 4 to 8 months of age they developed aggressive T cell lymphomas that displayed a high frequency of chromosome 15 trisomy. These results indicate that DNA hypomethylation plays a causal role in tumor formation, possibly by promoting chromosomal instability.

Human cancer cells often display abnormal patterns of DNA methylation. The role of aberrant hypermethylation in the silencing of tumor suppressor genes is now well documented (*1*). In contrast, the role of aberrant hypomethylation—which is observed in a wide variety of tumors (*2*–*5*), often together with regional hypermethylation—has remained unclear.

To investigate whether DNA hypomethylation has a causal role in tumor formation, we generated mice with highly reduced levels of Dnmt1, the enzyme that maintains DNA methylation patterns in somatic cells (*6*). Because mice homozygous for a Dnmt1 null allele ($Dnmt1^{c/c}$) die during gestation (*7*, *8*), we combined a hypomorphic allele [$Dnmt1^{chip}$ (*9*)] with a null allele to generate $Dnmt1^{chip/c}$ (referred to here as $Dnmt1^{chip/-}$) compound heterozygotes with a substantially reduced level of genome-wide DNA methyl-

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